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原　著

Ultrastructural Alterations of the Nerve Cell Bodies in the Dog Superior Cervical Ganglion, Following Surgical Destruction of the Hypothalamus

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Abstract

The hypothalamic locus between the infundibulum and the mamillary body of the dog was destructed by means of electrocoagulation, and the changes in the nerve cell bodies of the surperior cervical ganglia were examined with an electron microscope during the period from 3 to 14 days after the operation.

The results show that the intranucleolar lighter zone of the sympathetic postganglionic neurons in the dogs with the hypothalamus destructed was consistently more enlarged than in the control dogs (normal untreated, craniectomized only, and temporal lobe-destructed). Also, the perikaryal lysosomes in the neurons increased greatly in number in the dogs subjected to the hypothalamic destruction.

The significance of these observations is discussed in relation to the presumable functional disturbances of the sympathetic postganglionic neurons due to the hypothalamic lesions.

Introduction

The hypothalamus, one of the important common structures in the brain of the vertebrates, has been known to extert a powerful control over the autonomic nervous system[17,18,19], including the one over the cerebral vasomotor function[12,31]. In view of the fact that the cerebral arteries, those in the extracerebral portions at least, are innervated by the vasoconstrictor fibers originated from the superior cervical ganglion[6,13,15,16,24,26], and that a sympathetic center exists in the posterior part of the hypothalamus[9,29], an attempt has been made in the present study to search for ultrastuctural changes in the sympathetic postganglionic neurons in the dogs after experimental destruction of the hypothalamus.

Key Words : superior cervical ganglia, hypothalamus, nucleolus, lysosome.
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Materials and Methods

Thirty-one mongrel dogs, each belonging to one of 3 age groups, 1 month, 3 months, and 1-4 years (adult), were used in this study. For the surgical destruction of the dog hypothalamus, the transbuccal direct vision was allowed to use[23]. The operation was done as follows : The jaws of the animal, which was in supine position, were held open under pentobarbital sodium anesthesia, and the soft palate was incised in the midline, using the electrosurgical unit. At the base of the skull between the hamuli pterygoidei a chisel was used to open a hole 1×0.5cm in size. This field of ventral vision was enough to show the dog hypothalamus, and the lesions were made by means of electrocoagulation (40 mA for 10sec.) of the locus between the infundibulum and the mamillary body, i. e. the posterior part of the hypothalamus (Fig. 1).

A total of the 20 dogs which survived the above operation for 3 to 14 days were further processed for the electronmicroscopical investigation of the superior cervical ganglia.

Nine untreated normal dogs, together with 2 sham-operated dogs on which only a craniectomy or an electrocoagulation of a part of the temporal lobe was performed, served as controls for the experiment.

The superior cervical ganglia were perfusion-fixed with 1.25% gluteraldehyde and 8% paraformaldehyde in M/15 phosphate buffer at pH 7.4 applied through the common carotid artery[27] for 2 hours. The ganglia were then removed from the dog and cut into small blocks, and then osmicated in 1% osmium tetroxide solution for 2 hours. From some ganglia, thin slices (ca. 100μ in thickness) were prepared on a Sorvall Tissue Sectioner for the purpose of demonstrating lysosomal acid phosphatase activity using a lead phosphate method[8].

All samples were dehydrated in graded series of ethanol and embedded in Epon 812. Thin sections were stained with uranyl acetate followed by lead citrate[30], and examined in JEOL 100B electron microscope.

Quantitative estimations were made of the perikaryal cytoplasmic volume occupied by lysosomes as well as of the volume of the lighter zones of the nucleoli in the dog sympathetic postganglionic neurons. This was done by means of weighing the cut-pieces of the relevant areas shown by the 20 electron micrographs each from the experimental and control dogs of age groups, 1 month, 3 months, and adult.

Observations and Results

A remarkable difference according to the postnatal age exists with respect to the size of the perikarya and their content of cell organelles in the dog superior cervical ganglion. Thus, the ganglionic nerve cell bodies in normal dogs at the age of 1 month (Fig. 2 left) are smaller in size than those of adults and contain a few lysosomes, which occupy on the average $0.90 \pm 0.18\%$ of the volume of the total perikaryal cytoplasm (Fig. 6 left). Two nucleoli may occur in a single nerve cell nucleus of a dog of 1 month of age, but only one nucleolus is usually present in the nerve cells of adult dogs.

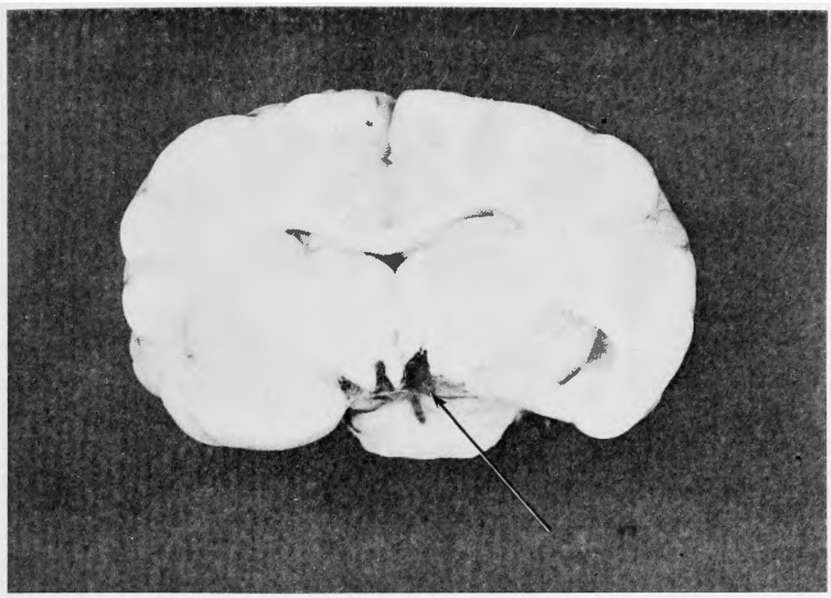

Fig. 1. A frontal section of the brain of a dog, showing the bilateral hypothalamic lesion (arrow) on the 14th day after the operation.

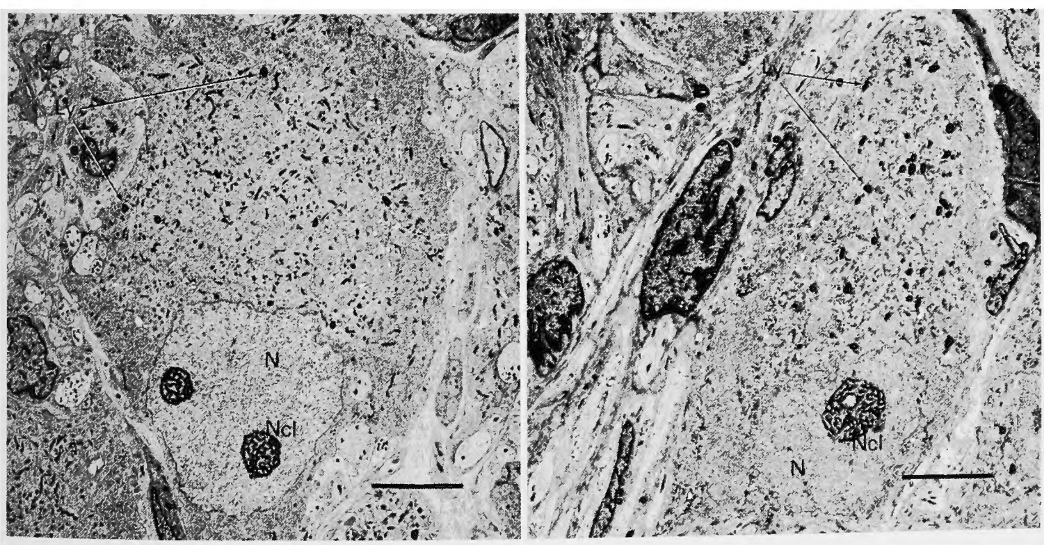

Fig. 2. Electron micrographs of the nerve cell bodies in the superior cervical ganglion at the age of 1 month. Left : From an unoperated control dog. The neuron soma has only a few lysosomes (Ly) within the cytoplasm. Right : On the 7th day after the hypothalamic destruction, the intracytoplasmic lysosomes are more numerous than in the control. Nucleolus(Ncl). Nucleus(N). Calibration, 5μ.

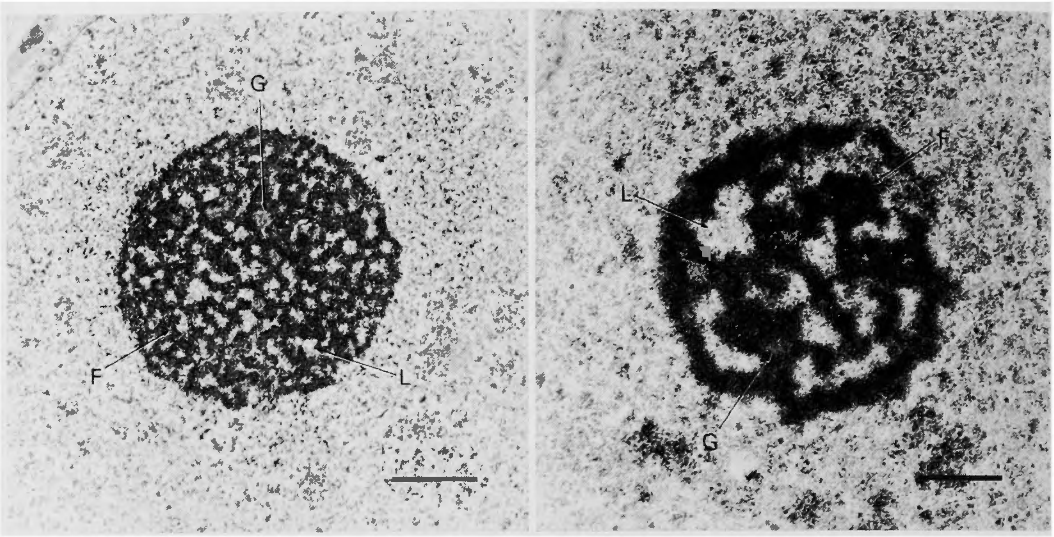

Fig. 3. Nucleoli of the sympathetic postganglionic neuron of dogs at the age of 1 month. Left : Normal nucleolus shows an intensely tangled pattern made by the pars granulosa (G) and pars fibrosa (F). Lighter zones (L). Right : The nucleolus from hypothalamus-destructed dog (7th day after the operation) shows a marked expansion of the intranucleolar lighter zones (L) and a much simpler arrangement of the nucleolar components. Calibration, 1μ.

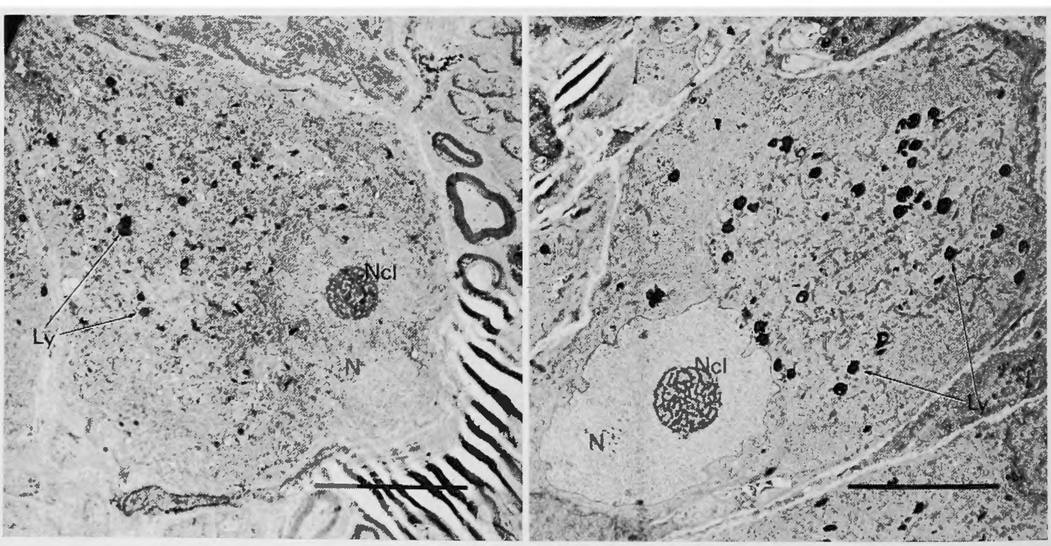

Fig. 4. The nerve cell bodies in the superior cervical ganglion of adult dogs. Left : From a normal control dog. The neuron soma shows a fair number of lysosomes (Ly) present in the perikaryal cytoplasm. Right : The neuron soma on the 7th day after hypothalamic destruction shows much more numerous lysosomes (Ly) than in the control. Nucleolus (Ncl). Nucleus (N). Calibration, 5μ.

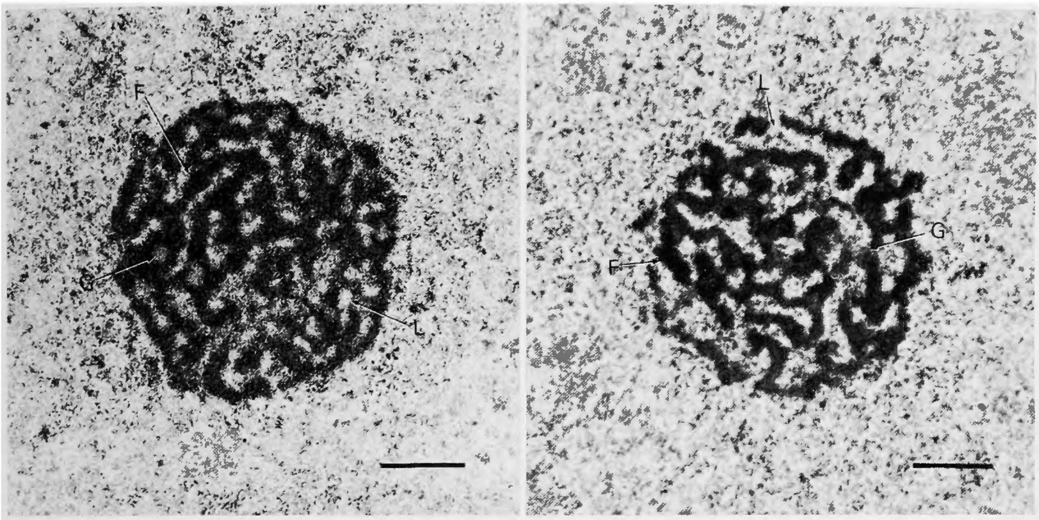

Fig. 5. The nucleoli in the superior cervical ganglia of adult dogs. Left : From a normal control dog. The nucleolus shows an intensive tangle of the partes granulosae (G) and fibrosae (F). Right : The nucleolus on the 7th day after hypothalamic destruction. The lighter zones (L) are much expanded and the nucleolar composition is crumbling. Calibration, 1μ.

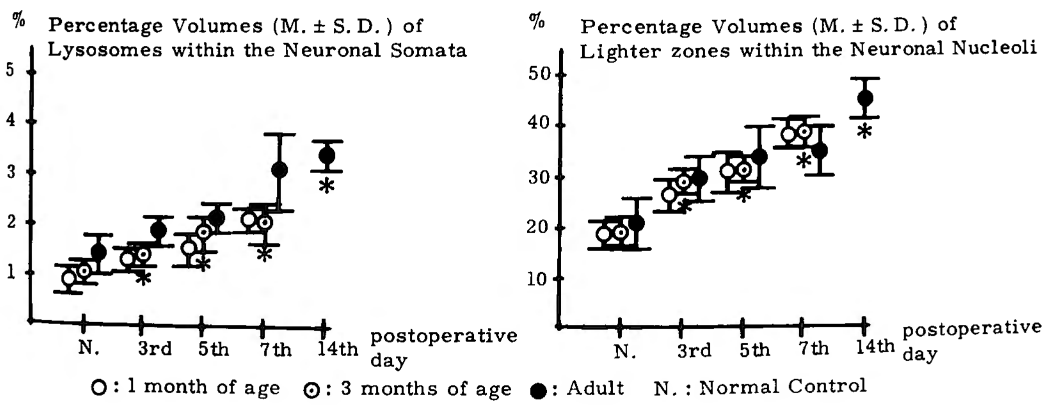

Fig. 6. Percentage volumes of the lysosomes within the neuronal somata (left) and of lighter zones within the neuronal nucleoli (right) under normal and experimental circumstances. The hypothalamic lesions seem to have caused an increase of the intraneuronal lysosomes as well as an enlargement of the lighter zones within the neuronal nucleoli, at least until the 14th day after the hypothalamic destruction. Open circles indicate the mean volume ± standard deviation for the dogs at the age of 1 month, dotted circles for the dogs at the age of 3 months, black circles for adult (over 1 year old) dogs. * : The difference in mean volume is statistically significant when compared to normal control volume ($P<0.001$, $N=38$).

In the case of the 1 month old dogs which received the hypothalamic destruction, however, the lysosomes in the nerve cell bodies of superior cervical ganglia became much more numerous (Fig. 2 right) than in the control, so as to occupy 1.32 ± 0.17% of the volume of the total perikaryal cytoplasm on the 3rd postoperative day, 1.56 ± 0.28% on the 5th, and 2.15 ± 0.20% on the 7th (Fig. 6 left).

In the control dogs 1 month of age, the nucleolar principal components, pars granulosa and pars fibrosa are evident and between the two components the lighter zones occupy on an average 18.96 ± 1.17% of the volume of the total nucleolar areas (Figs. 3 left and 6 right).

Following the hypothalamic destruction, the intranucleolar lighter zones became much more expanded, and also, the tangling pattern of the nucleolar components became much simpler postoperatively than in the unoperated dogs (Fig. 3 right), so as to account for 26.35 ± 1.99% of the volume of the total neuron nucleolus on the 3rd postoperative day, 31.13 ± 3.16% on the 5th, and 37.65 ± 2.00% on the 7th (Fig. 6 right).

In unoperated normal adult dogs, the superior cervical ganglia contain neuron somata of various sizes. Whithin the perikaryal cytoplasm, the lysosomes occur in fairly large numbers, occupying 1.45 ± 0.32% of the volume of the total perikaryal cytoplasm (Figs. 4 left and 6 left), but again became much more numerous after the hypothalamic lesions, so as to occupy 1.92 ± 0.24% of the volume of the total perikaryal cytoplasm on the 3rd postoperative day, 2.15 ± 0.26% on the 5th, 3.13 ± 0.75% on the 7th, and 3.40 ± 0.25% on the 14th (Fig. 6 left).

The pars granulosa and pars fibrosa in the neuronal nucleolus of the normal adult dogs are intensively tangled together and the development of the lighter zones is almost of the same order as in the normal puppy of 1 month of age, in which they occupied 20.96 ± 4.67% of the volume of the total neuronal nucleolus (Figs. 5 left and 6 right).

In the case of the hypothalamic destruction, the intranucleolar lighter zones in the sympathetic postganglionic neurons of the adult dogs became much larger, so as to account for 29.24 ± 4.43% of the volume of the total neuronal nucleolus on the 3rd postoperative day, 33.81 ± 6.04% on the 5th, 35.00 ± 4.97% on the 7th, and 45.83 ± 3.96% on the 14th (Fig. 6 right), and the tangling patterns of the nucleolar partes granulosa and fibrosa became much simpler than in the normal control (Fig. 5 right).

The same tendency was also noted in the dogs 3 months of age (Fig. 6).

Thus, the hypothalamic lesions appear to have caused an increase of the intraneuronal lysosomes and, at the same time, caused an enlargement of the lighter zone in the neuronal neucleoli at least until the 14th day after the operation.

These particular ultrastructural chnges in the sympathetic postganglionic neurons were not observed in the control animals, which received lesions of the temporal lobe or were subjected to the sham-operation.

Discussion

The present study indicated that some ultrastructural changes, which are most probably due to the hypothalamic lesions, occur in the sympathetic postganglionic neurons especially in their lysosomes and nucleoli. With the advent of electron microscopy, it became possible to define nucleolar components in a much more satisfactory way than had been possible with light microscopy. Studies on the nucleolar ultrastructure have shown that the nucleolus lacks a definite membrane but contains dense granular elements and fibrillar elements which have a density that differs from that of the remainder of the nucleolus, the lighter zones[3,28]. Its granular components, according to the majority of ultrastructural studies, including ultrastructural cytochemical observation[21], seem to be related to the formation of cytoplasmic ribosomes and may be their precursors. JONES[14] supported this concept by showing that nucleoli were compact and lacked granular components in the cells in which there was no synthesis of ribosomal RNA. The granular components are similar in some respects to cytoplasmic ribosomes and they probably contain 28S RNA. Correlative biochemical and ultrastructural studies provided evidence that some 4-7S RNA is probably present in the fibrillar components[3]. However, some fibrillar components may represent sites of synthesis of precursors for granular components or may contain messenger RNA[3]. Furthermore, morphological as well as cytochemical studies on the nucleoli in nerve cells under different experimental and pathological conditions have shown that the neuronal nucleolus is highly responsive to cell reactions and hence, is an important indication of the cell's function[4,11]. So, it seems that the postoperative changes observed in the present study in the ratio of the two components to the lighter zones in the nucleoli may suggest an alteration, somehow, of the postganglionic cell activities caused by the hypothalamic destruction.

The lysosomes are to a pertinent correspondence against various kinds of attacks from the outside or to the intracellular changes resulting from them[5]. As a matter of fact, alterations in lysosomal size and distribution have been previously noted in injured neuronal perikarya and axons[2]. Also, large numbers of autophagic vacuoles have been known to appear in the perikarya of neurons in response to mechanical injury[10], radiation[7], glucose starvation[25] and injection of colchicine[32]. As regards the postoperative increasement of the neuronal lysosomes observed in the present study, it can be suspected that any regressive changes had happened in the sympathetic postganglionic neuronal cytoplasm due to destruction of hypothalamus. In view of the general tendency that in the process of normal aging, the lysosomes in cells increase in number[28], changes in lysosomes in response to hypothalamic destruction were assessed in comparison with normal dogs belonging to the same age group.

Concerning fiber connections of the posterior hypothalamus, BEATTIE et al[1]. noted in degenerative experiments using cats that there were some direct connections between the posterior part of the hypothalamus and the lateral grey columns of the spinal cord, but this observation has not been universally accepted[22]. MAGOUN et al[20]. stated that hypothalamic efferents must descend, probably by a series of relays, through fibers scattered in the reticular formation of the mid-brain and pons, and then to the anterior and lateral white columns of the cord. At least a part of these hypothalamic pathways are said to decussate, and for this reason the posterior hypothalamus was destructed bilaterally in the present study.

The postoperative changes were consistent in the superior cervical ganglia of both sides. It seems, however, that further studies are needed to determine the effects of unilateral destruction of the hypothalamus on a single sympathetic ganglion.

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References

1) Beatte, J., Brow, G. R. and Long, C. N. H. : Physiological and anatomical evidence for the existence of nerve tracts connecting the hypothalamus with spinal sympathetic centres. Pro. Roy., Series B **106** : 253, 1930.
2) Becker, N. H. and Barron, K. D. : The cytochemistry of anoxic and anoxic-ischemic encephalopathy in rats. Am. J. Pathol., **38** : 161, 1961.
3) Busch, H. and Smetana, K. : The Nucleolus. New York Academic Press Inc., 1970.
4) Cook, W. H., Walker, J. H. and Barr, M. L. : A cytological study of transneuronal atrophy in the cat and rabbit. J. Com. Neurol., **94** : 267, 1917.
5) Deduve, C. and Wattiaux, R. Functions of lysosomes. Ann. Rev. Physiol., **28** : 435, 1966.
6) Falck, B., Nielsen, K. C. and Owman, C. : Adrenergic innervation of the pial circulation. Scand. J. Lab. Clin. Invest. Suppl., **102** VI : B, 1968.
7) Forssmann, W. G., Tinguely, H. and Posternak, J, M. . L'Ultrastructure du ganglion cervical supérieur du rat. Z. Zellforsch., **72** 325, 1966.
8) Gomori, G. : An improved histochemical technic for acid phosphatase. Stain Tech., **25** : 81, 1950.
9) Hess, W. R. : Hypothalamus und Thalamus. Stuttgart Georg Thieme, 1956.
10) Holtzman, E. and Albala, A. : Autophagic vacuoles and other lysosomes in the rat ganglion nodosum. J. Cell Biol., **31** 49A, 1966.
11) Hydén, H. Protein metabolism in nerve cell during growth and function. Acta Physiol. Scand., **6** : 1, 1943.
12) Ishii, S. : Brain swelling : studies of structural, physiological and biochemical alterations, in Caveness, W. H. and Walker, A. F. (eds) : Head Injury Conference Proceedings, Philadelphia and Toronto Lippincott., 276, 1966.
13) Iwayama, T., Furness, J. B. and Burnstock, G. : Dual adrenergic and cholinergic innervation of the cerebral arteries of the rat. Circul. Res., **26** : 635, 1970.
14) Jones, K. W. : The role of the nucleolus in the formation of ribosomes. J. Ultrastruct. Res., **13** : 257, 1965.
15) Kajikawa, H. : Fluorescence histochemical studies on the distribution of adrenergic nerve fibers to intracranial blood vessels. Arch. Jap. Chir., **37** : 473, 1968.
16) Kajikawa, H. : Mode of the sympathetic innervation of the cerebral vessels demonstrated by the fluorescent histochemical technique in rats and cats. Arch. Jap., Chir., **38** : 227, 1969.
17) Karplus, J. P. and Kreidl, A. : Gehirn und Sympathicus : I. Zwischenhirnbasis und Halssympathicus. Pflügers Arch. Ges. Physiol. **129** : 138, 1909.
18) Karplus, J. P. and Kreidl, A. : Gehirn und Sympathicus . II. Ein Sympathicuszentrum im Zwischenhirn. Pflügers Arch. Ges. Physiol., **135** : 401, 1910.
19) Karplus, J. P. and Kreidl, A. : Gehirn und Sympathicus : III. Sympathicusleitung im Gehirn und Halsmark. Pflügers Arch. Ges. Physiol., **143** : 109, 1912.
20) Magoun, H. W., Ranson, S. W. and Hetherington, A. : Descending connections from the hypothalamus. Arch. Neurol. Psychiat. Chicago, **39** : 1127, 1938.

21) Marinozzi, V. : Cytochemie ultrastructurale de nucléole —— RNA et protéines intranucléolaires. J. Ultrastruct. Res. **10** : 433, 1964.
22) Mitchell, G. A. G. : Anatomy of the Autonomic Nervous System. London E. and S. Livingston Ltd., 1953.
23) Nagamachi, Y. : Experimental procedures for dog's hypothalamus and hypophysis by the method of transbuccal direct vision. Clinical Physiol., **1** : 367, 1971 (Jap).
24) Nelson, E. and Rennels, M.: Innervation of intracranial arteries. Brain, **93** : 475, 1970.
25) Nicolescu, P., Dolivo, M., Rouiller, C. and et al. : The effect of deprivation of glucose on the ultrastructure and function of the superior cervical ganglion of the rat in vitro. J. Cell Biol., **29** · 267, 1966.
26) Nielsen, K. C. and Owman, C. : Adrenergic innervation of pial arteries related to the circle of Willis in the cat. Brain Res., **6** : 773, 1967.
27) Palay, S. L., McGee-Russell, S. M., Gordon, S. Jr., and et al. : Fixation of neural tissues for electron microscopy by perfusion with solutions of osmium tetroxide. J. Cell Biol., **12** : 385, 1962.
28) Peter, A., Palay, S. L. and Webster, H. F. : The Fine Structure of the Nervous System. New York Harper and Row Publishers, 1970.
29) Ranson, S. W. and Magoun, H. W. : The hypothalamus. Ergebn. Physiol., **41** : 56, 1939.
30) Reynolds, E. S. : The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol., **17** : 208, 1963.
31) Stornelli, S. A. and French, J. D. : Subarachnoid hemorrhage——factors in prognosis and management J. Neurosurg., **21** : 769, 1964.
32) Wiśniewski, H. and Terry, R. D. : Experimental colchicine encephalopathy. Lab. Invest. **17** : 577, 1967.

和文抄録

犬視床下部破壊時における上頸神経節神経細胞の微細構造変化について

岩手医科大学脳神経外科学教室（主任：金谷春之教授）

長野隆行

I. 実験目的

視床下部は現在，種々の実験により交感神経系と副交感神経系の中枢として自律機能の最高の座と考えられている．しかし視床下部と自律神経系の末梢部との関係を実験形態学的に追究した例はほとんどなく，そこで本研究においては視床下部の実験的破壊による交感神経節ニューロンへの微細構造変化を電顕的に追究した．

II. 実験方法

実験動物として生後1ヶ月，3ヶ月及び1才以上の雑種犬31頭を用いた．視床下部破壊には経口蓋法による犬の視床下部実験法を用い，破壊には直径約0.5mmの鋼線を使用し約40mAで10秒間電流を通して行った．視床下部破壊後，3日目，5日目，7日目及び14日目に両側頸動脈より，pH7.4に緩衝した1.25% Gluteraldehyde液で注入固定を行い，上頸神経節を採取，電顕用標本を作製し観察に用いた．又，正常無処置犬各年令シリーズ2頭づつ，及び側頭葉破壊のもの2頭，頭蓋底に骨窓形成したのみのもの2頭についての観察をも平行して行い視床下部破壊実験に対する対照所見とした．観察は各シリーズ20枚づつの電顕写真の神経細胞に対するlysosomeの比，及び核小体全体に対するその構成成分の1つであるlighter zoneの比をpaper-weight法により測定した．

III. 実験結果

1. 神経細胞に対するlysosomeの比：
 1) 生後1ヶ月
 正常犬 0.90±0.18%，視床下部破壊後3日目 1.32±0.17%，5日目 1.56±0.28%，7日目 2.15±0.20%
 2) 生後3ケ月
 正常犬 1.02±0.21%，破壊後3日目 1.48±0.22%，5日目 1.87±0.25%，7日目 2.08±0.17%
 3) 生後1才以上（成犬）
 正常犬 1.45±0.32%，破壊後3日目 1.92±0.24%，5日目 2.15±0.26%，7日目 3.13±0.75%，14日目 3.40±0.25%
2. 核小体に対するlighter zoneの比：
 1) 生後1ケ月
 正常犬 18.96±1.17%，破壊後3日目 26.35±1.99%，5日目 31.13±3.16%，7日目 37.65±2.00%
 2) 生後3ケ月
 正常犬 19.34±1.82%，破壊後3日目 28.34±1.57%，5日目 31.45±1.79%，7日目 38.74±2.52%
 3) 生後1才以上
 正常犬 20.96±4.67%，破壊後3日目 29.24±4.43%，5日目 33.81±6.04%，7日目 35.00±4.97%，14日目 45.83±3.96%

以上の実験結果より視床下部破壊により，上頸神経節神経細胞の特にlysosomeと核小体に関して変化が起ることが明らかとなった．交感神経系は一般に脊髄内側柱に細胞体をもつ節前ニューロンと交感神経節内細胞体をもつ節後ニューロンの二者によって構成されていることはよく知られているところであるが，本実験結果よりすくなくとも視床下部から節前ニューロン細胞体までの連絡が断たれたための影響が節後ニューロンの活動に及んでいることを示していると考えられる．